

=Electronic Supplementary Information=

An *Escherichia coli* trap in human serum albumin microtubes

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Experimental

Materials and Apparatus

All reagents were purchased from commercial sources as special grades and used without further purification. Human serum albumin (HSA) was purchased from Japan Blood Products Org. Poly-L-arginine hydrochloride (PLA, Mw: ca. 70 kDa), poly-L-glutamic acid sodium salt (PLG, Mw: 50–100 kDa), and poly(sodium 4-styrenesulfonate) (PSS, Mw: ~70 kDa) were purchased from Sigma-Aldrich Corp. *Escherichia coli* (*E. coli*) K12 (XL10-gold) was purchased from Agilent Technologies Japan, Ltd. The Fe₃O₄ nanoparticles (ca. 10 nm) covered with cationic surfactant (EMG607, 100 mg/mL) were purchased from Ferrotec Corp, Japan. UV-Vis absorption spectra were recorded using a UV-visible spectrophotometer Lambda Bio+ (PerkinElmer Co., Ltd.). The water was deionized (18.2 MΩcm) using water purification systems Elix UV and Milli Q Reference (Millipore Corp.).

Template Synthesis of (PLA/HSA)₉ MTs and (PLA/HSA)₇PLA/f-HSA/PLA/HSA MTs (fluorescent MTs)

The (PLA/HSA)₉ microtubes (MTs) were prepared according to our previously reported procedures with some modifications.⁹ The track-etched polycarbonate (PC) membrane (Isopore membrane, $r = 25$ mm, pore diameter (D_p) = 1.2 μm; Millipore Corp.) was placed into a stainless steel syringe holder (25 mm; Advantec Mfs, Inc.). Sodium phosphate buffer (PB) solution (pH 7.1, 10 mM, 10 mL) of PLA (1 mg/mL) containing 0.1 M NaCl was first passed through the membrane (0.25 mL/min) using a syringe pump (PHD-2000; Harvard Apparatus). The positively charged PLA was absorbed onto the negatively charged PC pore walls. Then excess PLA was washed away by water filtration (10 mL, 1.0 mL/min) and the membrane was dried *in vacuo* for 10 min. Second, the PB solution (pH 7.1, 10 mM, 10 mL) of HSA (2 mg/mL) was injected (0.5 mL/min) into the pores to create the second layer of negatively charged HSA. After water filtration (10 mL, 1.0 mL/min) to remove physically adsorbed protein, the membrane was dried under vacuum for 10 min. These pressure infiltrations were repeated for 9-cycles to grow the LbL thin film of (PLA/HSA)₉. The top and bottom surfaces of the PC membrane were wiped using a wet cotton swab and stored in refrigerator. To liberate the protein MTs from the template, the

membrane was immersed into a DMF solution and the precipitates were freeze-dried *in vacuo*, yielding the (PLA/HSA)₉ MTs as white powder.

The fluorescent microtubes, (PLA/HSA)₇PLA/f-HSA/PLA/HSA MTs, were prepared by the same procedure using fluorescein-labeled HSA (f-HSA) as the 16th layer. Similar microtubes, (PLA/HSA)₈PLA/PLG MTs and (PLA/HSA)₈PLA/PSS MTs, were also prepared by the same procedure using PB solutions (pH 7.1, 50 mM, 10 mL) of PLG and PSS.

Synthesis of Fe₃O₄(PLA/HSA)₉ MTs

Magnetic protein MTs were synthesized as follows. The Isopore membrane ($r = 25$ mm, $D_p = 1.2$ μ m; Millipore Corp.) was set into a stainless steel syringe holder and the aqueous Fe₃O₄ nanoparticle solution (1 mg/mL, 10 mL) was first filtered (0.25 mL/min) three times using a syringe pump (PHD-2000). The membrane was removed from the holder and wiped both sides using a wet cotton-swab to remove adherent Fe₃O₄. The obtained pale-brown membrane was dried in an automatic low-humidity chamber Super Dry (Tokyo Living Co. Ltd., Japan) for 12 h (humidity < 1%). Subsequently, the (PLA/HSA)₉ layers thin-film was fabricated in the Fe₃O₄ treated PC membrane by the same procedure described above. To eliminate the MT cores from the template, the membrane was immersed into a DMF solution and the released cores were freeze-dried *in vacuo*, yielding the Fe₃O₄(PLA/HSA)₉ MTs as a dark-yellow powder. Applying the average thickness of a PLA/HSA bilayer (16.4 nm) in the (PLA/HSA)₉ MTs to the Fe₃O₄(PLA/HSA)₉ MTs, the exterior surface layer of Fe₃O₄ was estimated as *ca.* 7 nm.

SEM Observations

For scanning electron microscopic (SEM) measurements of the MTs, the lyophilized sample was fixed directly onto the carbon tape and sputter-coated with Pd–Pt using ion sputtering (E-1045; Hitachi Ltd.). The SEM observations were conducted using a field-emission scanning electron microscope (S-4300; Hitachi Ltd.) with accelerating voltage of 10 kV. For each sample, at least 40 different MTs were measured to obtain an average size of the outer diameter and wall thickness.

Swelling Ratio of PLA Layer (α_{PLA})

The swelling ratio of the PLA layer (α_{PLA}) defined as a ratio between the section area in swollen state and that in dried state was estimated by equation (1).

$$\sum_{k=1}^9 \left(\pi \left[\left\{ \frac{D_s}{2} - (k-1)T_{PLAs} - (k-1)T_{HSA} \right\}^2 - \left\{ \frac{D_s}{2} - kT_{PLAs} - (k-1)T_{HSA} \right\}^2 \right] \right) / \sum_{k=1}^9 \left(\pi \left[\left\{ \frac{D_d}{2} - (k-1)T_{PLAd} - (k-1)T_{HSA} \right\}^2 - \left\{ \frac{D_d}{2} - kT_{PLAd} - (k-1)T_{HSA} \right\}^2 \right] \right) \quad \dots (1)$$

where D_s and D_d respectively represent the outer diameters of the MTs in swollen and dried states, T_{HSA} is thickness of the HSA layer,^{15,16} and T_{PLAs} and T_{PLAd} respectively represent thicknesses of the PLA layers in swollen and dried states.

E. coli Trapping Capability of (PLA/HSA)₉ MTs and Fe₃O₄(PLA/HSA)₉ MTs

The lyophilized powder of the (PLA/HSA)₉ MTs (*ca.* 150 μ g) was dispersed in deionized water (0.7 mL) using bath-type sonication for a few second. To avoid an electrostatic or nonspecific interaction between the *E. coli* and tube's exterior surface, 1 mM HSA (0.2 mL) was added; the total HSA concentration became 0.2 mM. The saline solution of *E. coli* (1×10^8 CFU/mL, 100 μ L) was added to the sample, and the resultant mixture was incubated with gentle rotation using an MTR-103 rotator (AS ONE Corp.) at 25 °C. The concentrations of MT and *E. coli*. were 1.6×10^7 tubes/mL and 1.0×10^7 CFU/mL, respectively. The ratio of MT/*E. coli*. is 1.6. After 1, 15, and 30 min, the 10 μ L of the mixture was pipetted out and diluted with saline by 1/1,000. Then 50 μ L of this solution was spread onto an LB Agar plate, and subsequently cultured at 37 °C for 16 h. The number of colonies appeared on the plate [N_c (MT)] was counted. The relative experiments were always carried out at the same time with (PLA/HSA)₃ NTs and without tube, and the numbers of colonies on the plates [N_c (NT) and N_c (Control)] were determined. The colony incidence was defined as [N_c (MT) or N_c (NT)]/ N_c (Control) \times 100 (%), and the disappearance yield was defined as (100 – colony incidence) (%). The similar experiments were performed with Fe₃O₄(PLA/HSA)₉ MTs.

Confocal Laser Scanning Microscopy (CLSM) Observations

Fluorescein-labeled HSA (f-HSA) was prepared using FluoroSpin 498 Protein Labelling & Purification Kit (emp Biotech GmbH). 5-Cyano-2,3-ditolyl tetrazolium chloride stained *E. coli* (CTC-*E. coli*) was prepared using CTC Rapid Staining Kit for Microscopy (BS02) (Dojindo Laboratories, Inc.). CLSM measurements were performed using a laser scanning microscope LSM 510-ZEN208 (Carl Zeiss Co., Ltd.). A droplet of the aqueous solution of CTC-*E. coli* with (PLA/HSA)₇PLA/f-HSA/PLA/HSA MTs (fluorescent MTs) was placed on the slide glass and shielded with cover glass and applied directly to the observations (ex. 488 nm).

Cell Viability Measurements of *E. coli* in (PLA/HSA)₉ MTs

The lyophilized powder of the (PLA/HSA)₉ MTs (*ca.* 150 µg) was dispersed in deionized water (0.7 mL) using bath-type sonication for a few second. To avoid an electrostatic or nonspecific interaction between the *E. coli* and tube's exterior surface, 1 mM HSA (0.2 mL) was added; the total HSA concentration became 0.2 mM. The saline solution of *E. coli* (1×10^8 CFU/mL, 100 µL) was added to the sample, and the resultant mixture was incubated with gentle rotation using a rotator at 25 °C. After 30 and 60 min, the 190 µL of the mixture was pipetted out and cell viability was measured using Microbial Viability Assay Kit-WST (M439) (Dojindo Laboratories, Inc.). This assay depends on the cleavage of the tetrazolium salt WST to form a dark-red soluble formazan dye product via the action of mitochondrial succinate-tetrazolium reductase in viable cells. The quantity of formazan dye is proportional to the number of metabolically active cells. Formazan dye can be quantified by measuring its absorbance at 450 nm [$A(\text{MT})$] using a Microplate Reader iMark (Bio-Rad Laboratories, Inc.). The relative experiments were always carried out at the same time with (PLA/HSA)₃ NTs and without tube, and the absorption at 450 nm [$A(\text{NT})$ and $A(\text{Control})$] were determined. The cell viability was defined as [$A(\text{MT})$ or $A(\text{NT})$]/ $A(\text{Control}) \times 100$ (%).

Results

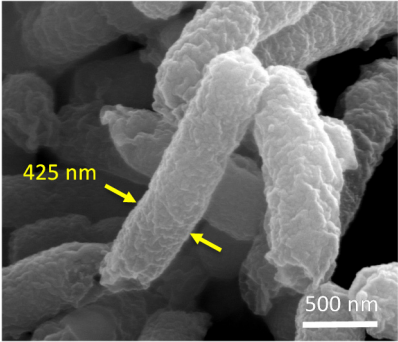


Fig. S1 SEM image of freeze-dried sample of *E. coli*. K12.

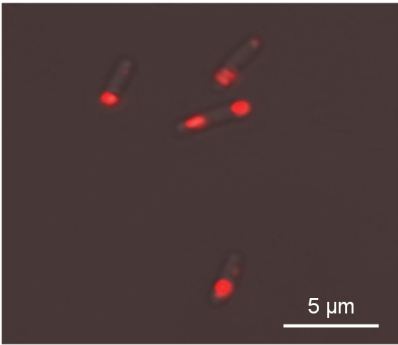


Fig. S2 Overlapping image of DIC and CLSM of CTC-*E. coli* (Ex. 488 nm).

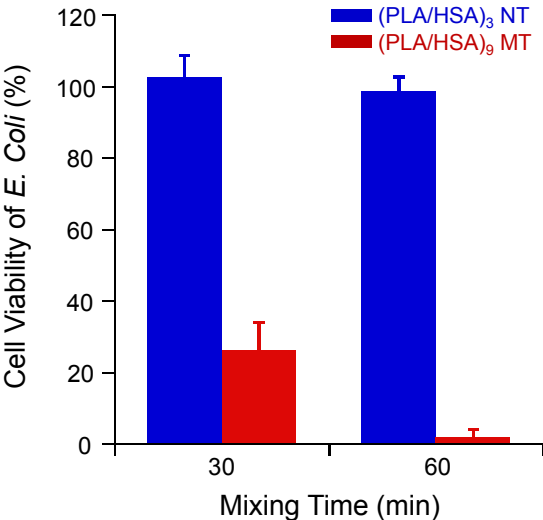


Fig. S3 Relation between mixing time and cell viability of *E. coli* ($n = 3$).